Characterization of the human cytochrome P450 enzymes involved in the metabolism of dihydrocodeine

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Aims Using human liver microsomes from donors of the CYP2D6 poor and extensive metabolizer genotypes, the role of individual cytochromes P-450 in the oxidative metabolism of dihydrocodeine was investigated.

Methods The kinetics of formation of *N*- and *O*-demethylated metabolites, nordihydrocodeine and dihydromorphine, were determined using microsomes from six extensive and one poor metabolizer and the effects of chemical inhibitors selective for individual P-450 enzymes of the 1A, 2A, 2C, 2D, 2E and 3A families and of LKM1 (anti-CYP2D6) antibodies were studied.

Results Nordihydrocodeine was the major metabolite in both poor and extensive metabolizers. Kinetic constants for *N*-demethylation derived from the single enzyme Michaelis-Menten model did not differ between the two groups. Troleandomycin and erythromycin selectively inhibited *N*-demethylation in both extensive and poor metabolizers. The CYP3A inducer, α-naphthoflavone, increased *N*-demethylation rates. The kinetics of formation of dihydromorphine in both groups were best described by a single enzyme Michaelis-Menten model although inhibition studies in extensive metabolizers suggested involvement of two enzymes with similar K_m values. The kinetic constants for *O*-demethylation were significantly different in extensive and poor metabolizers. The extensive metabolizers had a mean intrinsic clearance to dihydromorphine more than ten times greater than the poor metabolizer. The CYP2D6 chemical inhibitors, quinidine and quinine, and LKM1 antibodies inhibited *O*-demethylation in extensive metabolizers; no effect was observed in microsomes from a poor metabolizer.

Conclusions CYP2D6 is the major enzyme mediating *O*-demethylation of dihydrocodeine to dihydromorphine. In contrast, nordihydrocodeine formation is predominantly catalysed by CYP3A.

Keywords: dihydrocodeine, metabolism, human liver microsomes, CYP2D6, CYP3A

Introduction

Dihydrocodeine (DHC) is a semisynthetic opioid with similar structure to codeine. Despite many years of clinical use as an antitussive and analgesic, and contemporary use as a substitute drug for opioid addicts, little was known about its metabolism in humans until quite recently. Hufschmid *et al.* measured urinary metabolites of DHC following an oral dose of the drug [1]. The major urinary metabolite was dihydrocodeine-6-glucuronide which was excreted in similar quantities as unchanged drug. Approximately 12% of the dose was recovered as nordihydrocodeine, some of which was present as acid hydrolysable conjugates. Minor metabolites which each accounted for up to 2% of the dose were dihydromorphine and nordihydromorphine. The involvement of CYP2D6 was investigated by determining the urinary metabolic profile of DHC after administration of the CYP2D6 inhibitor quinidine. Almost no dihydromorphine or nordihydromorphine, both *O*-demethylated metabolites, could be detected after administration of quinidine 2 h prior to the DHC dose. Fromm *et al.* quantified DHC and dihydromorphine in plasma and nordihydrocodeine and dihydromorphine in urine to determine differences in DHC metabolism following an oral dose of the drug to poor and extensive metabolizers of sparteine/debrisoquine [2]. There was a significant difference in the amount of dihydromorphine formed in poor *vs* extensive metabolizers with this metabolite accounting for a mean of 9% of the dose recovered in urine of extensive metabolizers compared with less than 2% in poor metabolizers. No difference in conjugated and unconjugated forms of DHC and nordihydrocodeine was demonstrated between the two groups. These authors concluded that DHC *O*-demethylation was catalysed mainly by CYP2D6 although poor metabolizers still formed small amounts of dihydromorphine, suggesting the involvement of other P-450 enzymes in the formation of dihydromorphine [2].

Involvement of CYP2D6 in the *O*-demethylation of DHC may have clinical significance since dihydromorphine is a potent opioid agonist with similar opioid receptor binding affinity and potency to morphine [3, 4] and 100

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times the activity of DHC and its *N*-demethylated and glucuronidated metabolites, nordihydrocodeine and dihydrocodeine-6-glucuronide [4].

The present study was undertaken to characterize the formation kinetics of dihydromorphine and nordihydrocodeine in human liver microsomes. In addition, using a combined approach, the involvement of specific cytochrome P450 enzymes in the formation of these two metabolites was investigated in microsomes from CYP2D6 extensive and poor metabolizer liver, and using chemical and antibody inhibition studies.

Methods

Chemicals

Dihydrocodeine tartrate was donated by 3M Pharmaceuticals (Thornleigh, NSW, Australia). Traces of contaminants were removed by extraction of DHC, from an aqueous solution adjusted to pH 13.6 with potassium hydroxide, into diethyl ether. Pooled ether extracts of DHC were evaporated under nitrogen to produce a glassy residue which was dissolved in phosphoric acid (0.002%). This produced chromatographically pure DHC. This solution was then diluted with 0.1 M phosphate buffer to suitable concentrations for addition to microsomal incubations. Dihydromorphine hydrochloride and nordihydrocodeine were synthesized as described previously [4]. Other materials were obtained from the following sources: erythromycin, α-naphthoflavone, sulphaphenazole, coumarin and dithiocarb sodium (diethyldithiocarbamate sodium) from Sigma Chemical Co. (St Louis, MO, USA); furafylline and S(+)-mephenytoin from Salford Ultrafine Chemicals and Research Ltd (Manchester, UK); troleandomycin from Pfizer (Groton, CT, USA); quinidine sulphate from Merck (Darmstadt, Germany); and quinine hydrochloride from BDH Laboratory Supplies (Poole, UK). All other reagents were purchased from commercial sources and were of analytical or h.p.l.c. grade as appropriate.

Human liver tissue and preparation of microsomes

Liver tissue was obtained from seven individuals who underwent liver resection for hepatic tumours. Approval was obtained from the Royal Adelaide Hospital Research Ethics Committee and all donors gave written informed consent for their liver tissue to be studied. All tissue samples used for preparation of microsomes were normal on gross morphology. Liver and microsome samples were stored at −70° C until used. Donors ranged in age from 25 to 73 years; three were female and all had normal clinical chemistry and haematology prior to surgery except donor 15 with elevated alkaline phosphatase (477 u l^{-1}), alanine transaminase (122 u l^{-1}) and globulins (46 g l^{-1}), consistent with hepatocellular disease, and donor 31 with hypoalbuminaemia (33 g l^{-1}) and anaemia (haemoglobin 9.7 g l^{-1}). DNA was extracted from liver tissue for determination of *CYP2D6* genotype. *CYP2D6* alleles screened were *CYP2D6*1A*, *CYP2D6*4A*, *CYP2D6*4B*, *CYP2D6*4C*, *CYP2D6*4D* and *CYP2D6*5* [5, 6]. These alleles are known by the following trivial names: wild-type, *CYP2D6A*, *CYP2D6B*, K29–1 and *CYP2D6D* [7]. Genotype, corresponding to extensive metabolizer phenotype, determined for donors 15, 23 and 31 was *CYP2D6*1A/CYP2D6*1A* and for donors 21 and 22 was *CYP2D6*1A/CYP2D6*4A*. Donor 16 was classified as an extensive metabolizer from *in vitro* dextromethorphan kinetics [8]. Donor 24 was identified as a poor metabolizer with the genotype *CYP2D6*4A/CYP2D6*4A*.

Genetic analysis of the S-mephenytoin polymorphism (m1 and m2 mutations) was performed on DNA extracted from all liver samples excluding donor 16 [9]. No mutations of the *CYP2C19* gene were detected.

Microsomal fractions were prepared by differential centrifugation of liver homogenates using the method of Zanger *et al.* [10] with the following minor modifications. (±)-Dithiothreitol and phenylmethanesulphonyl fluoride were omitted from the preparation solution and the pH of this solution was adjusted to 7.3 by addition of sodium hydroxide; ethylene diamine triacetic acid (0.1 mM) was added to the sodium phosphate buffer used for storage of the microsomes. Microsomal protein concentrations were determined by the method of Lowry *et al.* [11] using bovine serum albumin (fraction V; Sigma Chemical Company) as the standard. Total cytochrome P-450 contents, determined by the method of Omura & Sato [12], ranged from 123 to 335 nmol g^{-1} protein. There was no evidence of reduced P-450 concentration (or activity measured by maximum formation rates of DHC metabolites) in microsomal samples from donors 15 and 31.

LKM1 antibody-positive serum was obtained from a donor with autoimmune chronic active hepatitis with an LKM1 antibody titre of 640. Control serum was obtained from a healthy volunteer. Serum protein concentrations were determined by the method of Lowry *et al.* [11].

Enzyme kinetic studies

The formation kinetics of nordihydrocodeine and dihydromorphine were studied by incubation of the substrate (DHC) at concentrations of 50 μM to 20000 or 24000 μM with microsomal protein (0.25 mg ml^{-1}) in a final volume of 200 μl containing an NADPH-generating system (1 mM NADP 1 unit ml^{-1} isocitrate dehydrogenase, 5 mM isocitric acid, 5 mM magnesium chloride) and 0.1 M sodium phosphate buffer (pH 7.4). The reaction was started by addition of microsomal protein and after 30 min at 37° C it was stopped by adding sodium carbonate (250 μl; 20% aqueous solution), vortex mixing and placing the tubes on ice. Linearity of the formation of metabolites with respect to protein concentration and incubation time was established with the given conditions. The formation rates of nordihydrocodeine and dihydromorphine were calculated as micromoles formed per minute per gram of microsomal protein (μmol $min^{-1}g^{-1}$).

Chemical inhibition studies

Single concentrations of each inhibitor were tested at a fixed substrate concentration (700 μM) which was in the vicinity of the calculated K_m for *O*-demethylation in three individual liver microsomal samples from extensive metabolizers (donor 16, 22 and 23) and at a substrate concentration of 6500 μM for the poor metabolizer sample (donor 24). The chemical inhibitors (and concentrations) tested were:

furafylline (5 μM), α-naphthoflavone (1 μM), coumarin (50 μM), sulphaphenazole (25 μM), S(+)-mephenytoin (100 μM), quinidine (0.2 μM), quinine (50 μM), ditiocarb (50 μM), troleandomycin (50 μM) and erythromycin (100 μM). Inhibitors were dissolved in water or dimethylsulphoxide (DMSO) in varying dilutions. An appropriate volume of the corresponding vehicle was added to each of the control incubations. Incubations were conducted as described above. The mechanism-based inhibitors, troleandomycin, erythromycin and furafylline, were pre-incubated with microsomes in the presence of NADPH-generating system for 15 min prior to addition of DHC and further NADPH-generating system. Pre-incubation was not used for α-naphthoflavone because the concentration of DMSO present resulted in excessive inhibition of microsomal activity during pre-incubation. Each inhibitor was incubated with microsomes in the presence of NADPH-generating system but absence of DHC to determine whether any interfering chromatographic peaks were produced.

The CYP2D6 inhibitor quinidine was studied further in microsomal samples from three extensive metabolizers (donor 16, 22 and 23) to determine the mechanism of inhibition and K_i (inhibition constant) values. Experimental conditions used were three DHC concentrations from 600 to 2000 μM and four quinidine concentrations from 0.02 to 0.2 or 0.3 μM. The same type of inhibition study was conducted for quinine (5 to 50 μM) in the same three livers.

LKM1 antibody inhibition studies

Inhibition by LKM1 antibody-positive serum was examined in microsomal samples from three extensive metabolizers (donor 16, 22 and 23) and one poor metabolizer (donor 24). Immunoinhibition studies were conducted by pre-incubating serum with microsomes for 10 min then adding the NADPH-generating system and substrate (700 μM). Corresponding control samples were prepared by pre-incubating control serum with microsomes prior to addition of NADPH-generating system and substrate.

Metabolite analysis

Nordihydrocodeine and dihydromorphine in microsomal incubations were quantified by h.p.l.c. Briefly, the method involved addition of sodium carbonate, cysteine and internal standard (cimetidine) to microsomal samples at the conclusion of incubations. This mixture was extracted with organic solvent (1-propanol : dichloromethane 1 : 9), the solvent evaporated, the residue reconstituted in 0.05% phosphoric acid and an aliquot injected onto a reversed phase h.p.l.c. column (4 μm Nova-Pak® phenyl 100 mm x 5 mm Radial-Pak™ cartridge from Waters Assoc. Milford, MA, USA). The mobile phase (acetonitrile : methanol : 0.05% phosphoric acid in water (pH 2.2–2.4) 10 : 5 : 85 v/v) was pumped at 1 ml min^{-1} and compounds detected at a wavelength of 210 nm. Assay performance was assessed by intra- and inter-day accuracy and precision for the analysis of quality control (QC) samples. Difference between calculated concentration and actual concentration and relative standard deviation was less than 15% at low QC concentrations and less than 10% at medium and high QC concentrations for both analytes.

Data analysis

The kinetic parameters of K_m (Michaelis-Menten constant), V_{max} (maximum velocity) and K_i were determined by non-linear regression analysis (Blackwell Regression, Blackwell Scientific Publications, Osney Mead, Oxford, U.K.) using untransformed, unweighted data.

For kinetic analysis in the absence of inhibitors, initial estimates of K_m and V_{max} were obtained from Eadie-Hofstee plots. Discrimination between kinetic models (single and two enzyme) in the absence of inhibitor was based on visual inspection of the graphical data (Eadie-Hofstee and Lineweaver-Burk plots), and review of the relative residuals and the standard error of the parameter estimates from non-linear regression analysis. Intrinsic clearance (CLint) was calculated as the ratio of V_{max} to K_m.

Models investigated for quinidine and quinine inhibition of *O*-demethylation of DHC included conventional relationships for competitive, uncompetitive and noncompetitive inhibition of a single enzyme and the apparent partial inhibition model (Equation 1), representing a two enzyme system in which the high-affinity enzyme was competitively inhibited and the low-affinity enzyme showed no inhibition [13].

$$\text{Equation 1:}\quad \text{Rate of metabolite formation} = \frac{V_{max1}.S/K_{m1}}{1 + S/K_{m1}} + \frac{V_{max2}.S/K_{m2}}{1 + S/K_{m2} + I/K_i}$$

where S is substrate concentration, I is inhibitor concentration, and V_{max1} and K_{m1}, and, V_{max2} and K_{m2} are the V_{max} and K_m of the uninhibited and inhibited enzymes, respectively. Dixon plots and slope replots, review of the relative residuals, the standard error of the parameter estimates, and how well the estimated K_m and V_{max} with inhibitor agreed with those estimated in the absence of inhibitor were used to assess appropriateness of the model.

For chemical inhibitors tested at a single concentration and LKM1 antibody inhibition, results are expressed as a percentage of control formation rates for each metabolite with mean ± s.d. for the three extensive metabolizer microsomal samples and a mean of replicate determinations ($n = 4$ for chemical inhibitors and $n = 2$ for LKM1 antibody inhibition) in the poor metabolizer sample.

Results

In vitro *metabolism of dihydrocodeine*

The microsomal reaction produced two major h.p.l.c. peaks with the same retention times as authentic nordihydrocodeine and dihydromorphine. There was no evidence of metabolite formation in microsomes incubated with DHC in the absence of NADPH-generating system.

N-demethylation to nordihydrocodeine was the predominant pathway at all substrate concentrations. In the six

extensive metabolizer and one poor metabolizer, Eadie-Hofstee plots of nordihydrocodeine formation were linear over the substrate concentration range of 400 to 24000 μM. In both extensive and poor metabolizers, the single enzyme model satisfactorily fitted the data (Table 1). The V_{max} and K_m in the poor metabolizer of 5.49 μmol $min^{-1}g^{-1}$ and 4351 μM, respectively, compared with mean values in extensive metabolizers of 3.86 μmol $min^{-1}g^{-1}$ and 9541 μM, respectively. The individual values for the extensive metabolizers ranged from 2.70–5.53 μmol $min^{-1}g^{-1}$ for V_{max}, and 5372–14194 μM for K_m.

Linear Eadie-Hofstee plots for *O*-demethylation of DHC were consistent with single enzyme kinetics in liver microsomes from both poor and extensive metabolizers. The kinetic parameters for this pathway in extensive metabolizers and the poor metabolizer are shown in Table 1. V_{max} for *O*-demethylation in both poor and extensive metabolizers was much lower than for *N*-demethylation. The V_{max} and K_m in microsomes from the poor metabolizer of 0.050 μmol $min^{-1}g^{-1}$ and 3235 μM, respectively, may be compared with the mean values in extensive metabolizers of 0.178 μmol $min^{-1}g^{-1}$ and 981 μM, respectively. The individual values for the extensive metabolizers ranged from 0.112–0.288 μmol $min^{-1}g^{-1}$ for V_{max}, and 692–1463 μM for K_m. In the poor metabolizer, the CLint was less than 10% of the mean value in the extensive metabolizer group.

Inhibition studies

Selective chemical inhibitors of various P-450 enzymes were tested for their ability to inhibit the metabolism of DHC in three extensive metabolizer microsomal samples and one poor metabolizer sample. The effect of each inhibitor on *O*- and *N*-demethylation pathways is shown in Figure 1. Troleandomycin and erythromycin caused substantial inhibition of *N*-demethylation in both poor and extensive metabolizer microsomes. α-Naphthoflavone induced nordihydrocodeine formation in extensive metabolizers however the effect was variable with a range of activity compared to controls of 124–300%. There was no apparent effect of α-naphthoflavone in microsomes from the poor metabolizer. There was no change in nordihydrocodeine formation rate demonstrated with the remaining compounds tested.

Quinidine and quinine were potent inhibitors of *O*-demethylation in extensive but not poor metabolizer microsomes (Figure 1). None of the remaining compounds inhibited dihydromorphine formation in either phenotypic group.

N-demethylation	K_m (μM)	V_{max} (*μmol* min^{-1} g^{-1})	*CLint* (*ml* min^{-1} g^{-1})
EM (mean ± s.d.; $n=6$)	9541 ± 3289	3.86 ± 1.12	0.478 ± 0.280
(range)	(5372–14194)	(2.70–5.53)	(0.213–0.857)
PM ($n=1$)	4351	5.49	1.26
O-demethylation			
EM (mean ± s.d.; $n=6$)	981 ± 282	0.178 ± 0.064	0.192 ± 0.075
(range)	(692–1463)	(0.112–0.288)	(0.095–0.277)
PM ($n=1$)	3235	0.050	0.015

Table 1 Kinetic parameters for dihydrocodeine *N*-demethylation and *O*-demethylation in CYP2D6 extensive (EM) and poor metabolizer (PM) liver microsomes.

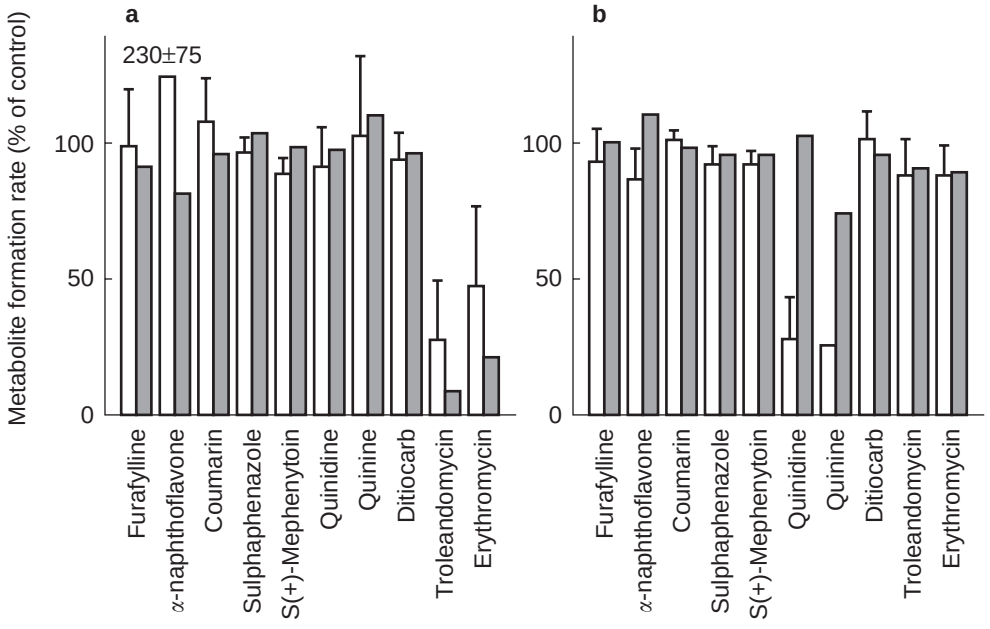

Figure 1 Effect of chemical inhibitors on dihydrocodeine *N*-demethylation (a) and *O*-demethylation (b) in CYP2D6 extensive metabolizer microsomes (open bars; mean ± s.d.; $n=3$) and poor metabolizer microsomes (shaded bars; $n=1$). Inhibitor concentrations used are specified under ***Methods***.

Table 2 Kinetic parameters and K_i (mean ± s.d.; $n = 3$) for inhibition of dihydrocodeine *O*-demethylation by quinidine in CYP2D6 extensive metabolizer liver microsomes.

K_{m1} (μM)	V_{max1} ($\mu mol\ min^{-1}\ g^{-1}$)	K_{m2} (μM)	V_{max2} ($\mu mol\ min^{-1}\ g^{-1}$)	K_i (μM)
3465 ± 382	0.042 ± 0.006	633 ± 312	0.159 ± 0.092	0.016 ± 0.012

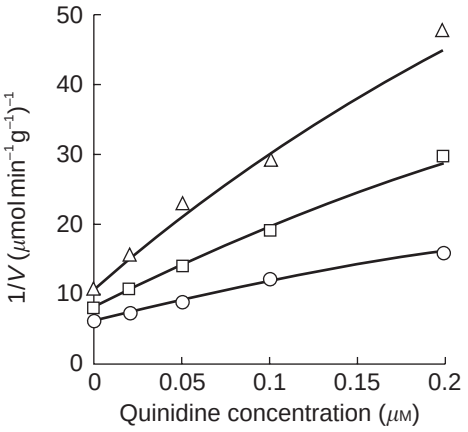

Figure 2 Representative Dixon plot for quinidine inhibition of dihydrocodeine *O*-demethylation in microsomes from a CYP2D6 extensive metabolizer. The line represents model fit for the apparent partial inhibition model. △ 600 μM DHC, □ 1000 μM DHC, ○ 2000 μM DHC.

The mechanism of inhibition of *O*-demethylation and K_i values were calculated for the CYP2D6 inhibitor quinidine in three extensive metabolizer microsomal samples. Dixon plots were curvilinear; a representative example (donor 22) is shown in Figure 2. The lines in Figure 2 are from the model which represents a two enzyme system in which the high affinity enzyme is inhibited competitively. The kinetic parameters for quinidine inhibition of *O*-demethylation are shown in Table 2. The calculated inhibition constant (mean ± s.d.) was 0.016 ± 0.012 μM. There was no effect on the rate of formation of nordihydrocodeine at the concentrations of quinidine used. The inhibition constant for quinine determined in the same three livers was 3.63 ± 0.54 μM.

LKM1 antibody-positive serum selectively inhibited dihydromorphine formation in microsomes from extensive metabolizers (Figure 3). Addition of 1 μl of serum to the incubation mixture produced maximal inhibition of 94% and higher concentrations (4 μl) did not produce any further inhibition. No inhibition of formation of either metabolite was seen in microsomes from the poor metabolizer.

Discussion

Recent studies of DHC metabolism *in vivo* in humans have identified the *N*- and *O*-demethylated metabolites, nordihydrocodeine and dihydromorphine, in urine and confirmed the presence of dihydromorphine in plasma [1, 2]. An important role for the polymorphically expressed CYP2D6 in *O*-demethylation to dihydromorphine has been demonstrated *in vivo* although involvement of other P-450 enzymes has also been suggested [1, 2]. Experiments using an *in vitro* animal model support this proposal [14]. There have been no reports of specific cytochrome P-450 enzymes involved in the *N*-demethylation pathway to nordihydrocodeine.

In this study of the oxidative metabolism of DHC in human liver microsomes we have confirmed formation of the metabolites nordihydrocodeine and dihydromorphine. Nordihydrocodeine was the major metabolic product measured in both extensive and poor metabolizers. This is consistent with the biotransformation of DHC *in vivo* where *N*-demethylation is the major route of oxidative metabolism and *O*-demethylation is a minor pathway [1, 2]. Kinetics of *N*-demethylation were not different between extensive and poor metabolizers and the single enzyme Michaelis-Menten model was appropriate to the experimental data. Involvement of CYP3A enzymes was evident from the inhibition of nordihydrocodeine formation following pre-incubation with the selective CYP3A inhibitor, troleandomycin and the CYP3A substrate, erythromycin [15, 16]. Additionally, α-naphthoflavone, which activates CYP3A4 [17], increased nordihydrocodeine formation. CYP3A4 catalysed *N*-demethylation is common to structurally related compounds, codeine [18], dextromethorphan [19] and ethylmorphine [20]. Lack of effect of a range of chemical inhibitors indicated that enzymes of other major P-450 subfamilies did not contribute significantly to the *N*-demethylation of DHC. The inhibitors tested (and their specific target cytochrome P-450 enzymes) were furafylline (CYP1A2), coumarin (CYP2A6), sulfaphenazole (CYP2C9), S(+)-mephenytoin (CYP2C19), ditiocarb (CYP2E1), quinidine and quinine (CYP2D6) [15, 21–23]. Inhibitor concentrations used in this study were in the range of concentrations shown previously to inhibit the respective enzymes.

Our results demonstrate that CYP2D6 is the main enzyme which catalyses *O*-demethylation of DHC. A small, but measurable, intrinsic clearance for the formation of dihydromorphine in microsomes from the poor metabolizer is likely to be contributed by other enzymes. The apparent affinity (K_m) of DHC (981 μM) for CYP2D6 in the absence of inhibitors in microsomes from extensive metabolizers is of the same order of magnitude as that reported for its structural congener codeine (149 μM) [24]. In microsomes from the poor metabolizer, the K_m for DHC (3235 μM) indicated a lower affinity enzyme was responsible as has been reported for codeine (> 1000 μM) [24]. The observed activity in extensive metabolizer microsomes is therefore likely to arise from contributions of at least two enzymes however the difference in their K_m values is not large enough for this to be evident from kinetic analysis of the data in the absence of inhibitor.

Inhibition of dihydromorphine formation by CYP2D6 chemical inhibitors, quinidine and quinine [23], supported involvement of CYP2D6 in extensive metabolizers. Consistent with the known relative inhibitory potencies of quinidine and quinine for CYP2D6 in humans [23], the inhibition constant for quinine was approximately 200-fold

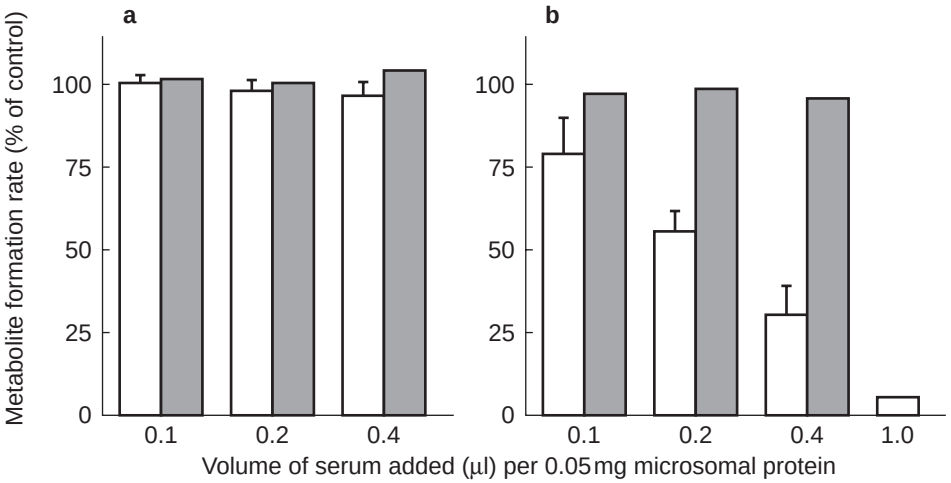

Figure 3 Effect of LKM1 antibody-positive serum on *N*-demethylation (a) and *O*-demethylation (b) of dihydrocodeine in CYP2D6 extensive metabolizer microsomes (open bars; mean ± s.d.; $n = 3$) and poor metabolizer microsomes (shaded bars; $n = 1$).

greater than that for quinidine. Quinidine has been shown to inhibit *in vivo* the *O*-demethylation of both DHC [1] and codeine [25, 26]. Quinidine inhibition studies in microsomes from extensive metabolizers substantiated the involvement of at least two enzymes since a model representing a two enzyme system in which one enzyme is selectively inhibited most satisfactorily described the data. Experiments using a selective CYP2D6 inhibitor would be expected to allow more sensitive detection of a two enzyme system; by inhibiting CYP2D6 catalysed metabolite formation the relative contribution of the other enzyme is increased. The mean apparent inhibition constant for quinidine (0.016 μM) is consistent with the known potency of this compound as a CYP2D6 inhibitor and is similar to that reported for codeine (0.015 μM) [24]. It is noted that the Michaelis-Menten constant of the high affinity enzyme (K_{m2}) in the presence of quinidine is similar to the apparent K_m in extensive metabolizers in the absence of inhibitor. Furthermore, K_{m1} is comparable with the K_m measured in the poor metabolizer without quinidine. Experiments with chemical inhibitors of CYP1A2, CYP2A6, CYP3A, CYP2C and CYP2E1 did not discriminate which other enzymes may be active for *O*-demethylation of DHC. LKM1 antibody-positive serum, which inhibits selectively CYP2D6 [27], produced concentration dependent inhibition of dihydromorphine formation in extensive metabolizers. Ten percent of activity was resistant to inhibition; a residual activity probably arising from the alternate enzyme or enzymes with catalytic activity for *O*-demethylation of DHC.

In agreement with previous studies conducted *in vivo* [1, 2] and experiments using a rat liver microsomal model [14], our investigations in human liver microsomes show that DHC undergoes oxidative metabolism to *N*- and *O*-demethylated metabolites, nordihydrocodeine and dihydromorphine, and that dihydromorphine formation is predominantly catalysed by CYP2D6. This latter conclusion is derived from the following: comparison of kinetic data between six extensive metabolizers and one poor metabolizer; almost complete inhibition of dihydromorphine formation by LKM1 antibody; and, the relative inhibitory potencies of quinidine and quinine. Additional supporting evidence may be provided from studies utilising expressed enzymes. A minor contribution to catalytic activity arises from at least one other unidentified enzyme. *N*-demethylation is the main route of oxidation and this pathway is catalysed by CYP3A enzymes. Clinical importance of CYP2D6 involvement in dihydromorphine formation is anticipated since dihydromorphine is approximately 100 times more potent than DHC and its other metabolites, nordihydrocodeine and dihydrocodeine-6-glucuronide [4]. Therefore, variable expression of the enzyme due to its polymorphic characteristics and potential drug interactions with other CYP2D6 substrates may result in an inconsistent response when DHC is administered in the clinical setting as a cough suppressant, analgesic or opioid drug substitute.

The authors wish to acknowledge the contributions of Mr J. A. R. Williams, Head of the Hepatobiliary and Pancreatic Surgery Unit, Royal Adelaide Hospital, who provided the liver tissue, Ms Heather James of the Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, and Ms Janet Coller of the Department of Clinical and Experimental Pharmacology, University of Adelaide, South Australia who genotyped the liver tissue and Professor Geoffrey Farrell, Head of the Department of Gastroenterology and Hepatology, Westmead Hospital, New South Wales, Australia who supplied the LKM1 antibody-positive serum.

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(*Received 21 April 1997, accepted 24 July 1997*)